Solution Equilibria between Aluminum(III) Ion and some Fluoroquinolone Family Members. Spectroscopic and Potentiometric Study

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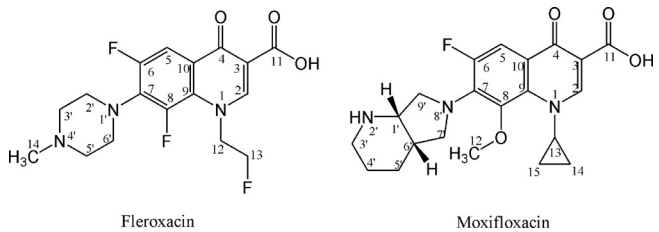
Complex formation between aluminum(III) ion and fluoroquinolone antibacterials-either moxifloxacin (4th generation antibiotic) or fleroxacin (2nd generation antibiotic) were studied in aqueous solutions without and in the presence of sodium dodecylsulfate (SDS). The investigations were performed by glass electrode potentiometric (ionic medium: 0.1 mol/dm^3 LiCl, 298 K), UV spectrophotometric, multinuclear (^{1}H and ^{13}C) magnetic resonance and ESI-MS measurements. The experimental data were consistent with the formation of Al(HL)L^{2+}, Al(HL)$^{3+}$, AlL^{2+}, Al(OH)L$^+$ and Al(OH)$_2$L complexes in the pH interval *ca.* 3—8 and up to 5 : 1 ligand to metal mole ratio with range of Al^{3+} concentrations between *ca.* 0.025 to 1.0 mmol/dm^3. The binary complex, AlL^{2+} is fairly stable (log $\beta_{1,0,1}$ *ca.* 11.0) and its stability increases in the presence of SDS. At higher concentration ratios of ligands to aluminum, up to 5 : 1, the complex Al(HL)L^{2+} is formed with rather high overall stability constant (log $\beta_{1,1,2}$ *ca.* 24.0). The ESI-MS data generally, confirmed the derived model, and the formation of the complex with ligand to metal ratio 2 : 1. NMR measurements indicate that both ligands utilize 4-carbonyl and carboxyl oxygens as donor atoms. The presence of surface active substance, SDS, favors the formation of the complex in which the ligand is protonated, *i.e.* Al(HL) and its maximum formation is shifted toward milder acidic region (pH *ca.* 4). The aluminum–quinolone complexes may affect the bio-distribution of both, quinolone and/or aluminum ion upon concomitant ingestion of aluminum-based antacids or phosphate binders and fluoroquinolones.

Key words aluminum; fleroxacin; moxifloxacin; complex formation; solution equilibrium

Fluorinated quinolones are antibacterial agents which chemically, may be regarded as weak substituted heterocyclic amino acids. These drugs primarily find use in the treatment of urinary and respiratory infections. Fluoroquinolones exhibit strong activity against Gram-negative and some Gram-positive bacteria, though many anaerobic strains are resistant.[1)] Fleroxacin and moxifloxacin are fluoroquinolone family members which belong to 2nd and 4th generation of these drugs, respectively (Chart 1).

In clinical practice often, fluoroquinolones are administered concomitantly with other drugs which may contain metal ions. The presence of metal ions from *e.g.* metal based antacids or multivitamin formulations may significantly affect the activity of quinolones since they can readily bind several divalent or trivalent metal ions.[2)] Complexation alters solubility, lypophilicity, antimicrobial activity and protein binding of quinolones. The solubility of all ionic quinolone complexes is much greater than that of molecular complexes which are only sparingly soluble.[3)] Some metal–quinolone complexes show antimicrobial activity comparable to that or free quinolone but in some cases the activity is increased or lowered. Mg^{2+} and Al^{3+} were found to decrease the activity of quinolones whereas Fe^{3+} and Zn^{2+} complexes exhibit greater activity.[2)] Clinical investigations have shown that concomitant intake of fluoroquinolones and aluminum-containing antacids results in reduced maximal plasma concentration accompanied by the decrease in *AUC*. Both effects lead to the decreased bioavailability of the drug, down to 40%.[4)] To explain the observed phenomenon one may take into account that number of forms in which quinolones may exist in solution (neutral, cationic, zwitterionic and anionic) as well as vicinity of carbonyl and carboxyl groups make them the suitable ligands for hard acid metal ions, in particular for Al^{3+}.[5—12)] On the other hand the Al^{3+} ion forms the complexes of the highest stability with the ligands containing hard donor groups (*i.e.* basic negatively charged oxygen atoms). Thus, the reduced bio-availability of the quinolones in the presence of Al may be explained by chelation between Al^{3+} ion and the 3-carboxyl and 4-oxo functional groups of the quinolones.[5—12)]



Fleroxacin Moxifloxacin

Chart 1

Besides the reduction of the bioavailability and activity of quinolones the interactions between aluminum ion and quinolones have significant effect on metabolism of aluminum in human organism. Numerous investigations have shown that aluminum can be regarded as detrimental and in particular neurotoxic element.[13)] Patients with high tissue and serum level of aluminum may develop blood, bone or brain diseases which may be linked to the excess of aluminum.[14)] The presence of fluoroquinolones may influence Al absorption through the formation of Al-complexes which may be stable enough to produce a sufficient decrease in free Al^{3+} concentration so that a significant amount of the poorly solu-

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ble aluminum compounds ($Al(OH)_3$, aluminum hydroxo-carbonates or $AlPO_4$) can dissolve and by formation of electrically neutral lipophile complexes may, cross gastrointestinal membrane by passive diffusion. The extent of its absorption into systemic circulation is primarily determined by the solubility of the ingested aluminum compound as well as by the complex formation with ligands present in intestinal secretions and the mucus, as well as other concomitantly ingested substances *e.g.* drugs.[15)]

Cellular uptake of aluminum–fluoroquinolone complexes is dependent on their interaction with membrane phospholipids so as to mimic their function, the effect of surfactant sodium dodecylsulfate (SDS) on complex formation between Al^{3+} and fleroxacin or moxifloxacin was studied. The surfactant would have the effect to solubilize both, the quinolone and its chelate complex with aluminum, to exclude water molecules from the complexation reaction sphere and to prevent the hydrolysis of Al–fluoroquinolone complexes.

The literature data on fluoroquinolones complexation have recently been reviewed.[16)] The complexation reactions, in solution, were studied between Al^{3+} ion and several fluoroquinolones: norfloxacin, lomefloxacin, ciprofloxacin and ofloxacin.[17—23)] The employed techniques of investigations of speciation in solution and stability of the formed complexes, involved spectrophotometry, potentiometry and multinuclear NMR. The stoicheiometry of the species formed in solution indicate the formation of the complexes with ligand to metal (L/M) ratios from 1 : 1 to 3 : 1. Also, various mixed hydrolytic and protonated complexes may be formed in solution. Generally, the complexes with Al^{3+} are more stable than those with divalent ions but their stability is lower than analogous Fe^{3+} chelates. It was proposed that quinolones interact with Al^{3+} in the stomach, but with Mg^{2+} in the intestines, when co-administered with antacid containing Al^{3+} and Mg^{2+}.

Thus, aluminum–quinolone interactions in biological systems have three important aspects: (a) reduced bio-availability, (b) altered activity of quinolone, and (c) toxic effects of aluminum ion. To give an explanation of these aspects it is necessary to study the speciation in the aluminum–quinolone systems so as to provide the reliable data on composition, stability and structure of the species formed in solution.

Still, however, the quantitative data concerning the complexation of quinolones with aluminum are rather scarce. The complexation between fluoroquinolones family members fleroxacin (2nd generation) and moxifloxacin (4th generation) and Al^{3+} ion was not studied so far neither the effect of micellar media on these complexation equilibria was examined. Therefore, the aim of the present paper is to quantitatively examine the equilibria in fleroxacin or moxifloxacin solutions in the presence of aluminum ion to gain better understanding of the interactions between aluminum containing drugs and these fluoroquinolones. The speciation model derived from such fundamental studies should help in pharmacokinetic studies of quinolones in the presence Al-containing drugs and also in the study of toxic effects of Al-ion upon concomitant intake of Al-containing drugs and fluoroquinolones.

Experimental

Reagents and Analysis The stock solution of aluminum(III) chloride was prepared by dissolving doubly recrystallized $AlCl_3\ 6H_2O$ p.a. (Merck) in twice distilled water. The appropriate amount of HCl was added to avoid initial hydrolysis of Al^{3+} ion. The aluminum content was determined gravimetrically by the precipitation either with 8-quinolinol or by ammonia. Both methods gave the same results within 0.3%. The concentration of the free acid was determined potentiometrically using the Gran plot. The constant of the total proton concentration with time was considered as a criterion for the absence of initial aluminum(III) hydrolysis and was periodically checked by titration against standard NaOH before each series of measurements.

Fleroxacin, [6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl piperazino)-4-oxo-3-quinoline carboxylic acid] standard (purity 100%, MW 369, white crystalline powder) was a product of Hoffmann La Roche (Basel, Switzerland). Moxifloxacin, [1-cyclopropyl-6-fluoro-8-methoxy-7-(4a*S*,7a*S*)-octahydropyrolo[3,4-b]pyridine-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride], (declared purity >99%), yellow powder, M_r= 437.9, was obtained from BayerPharma AG (Germany). Sodium *n*-dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3Na$, M_r=288.4, was product of Sigma (U.S.A.). Before use SDS was purified by washing with ether and 95% ethanol and subsequently dried in a desiccator containing P_2O_5. Purity was checked with TLC and spectrophotometry, according to recommended procedure and by measurement of pH of water solution. No basic impurities were detected.

The sodium hydroxide solution was prepared from concentrated volumetric solutions p.a. (Merck) by diluting with freshly boiled doubly distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogen phthalate. The hydrochloric acid solution was made from HCl "Suprapure" (Merck) and standardized against tris-(hydroxymethyl) aminomethane. The solution of lithium chloride was prepared from LiCl, p.a. (Merck) by dissolving the recrystallized salt in twice de-ionized water. The concentration was determined by evaporation of a known volume of solution to dryness at 573 K and weighing the residue.

Equipment Potentiometric measurements were carried out using a Tacussel (France) Isis 20000 digital pH-meter with a resolution ±0.1 mV (in some measurements an extended scale was used with a resolution ±0.01 mV). The pH meter was equipped with a Radiometer (Copenhagen, Denmark) GK2401B combined electrode. Titrant was delivered from a Metrohm (Donau, Swiss) Dosimat model 665 autoburette. The constant temperature was maintained with a VEB Prufgerate model E3E circulating ultrathermostat. Spectral measurements were made on double beam UV–Vis model Lambda 35 (Perkin Elmer, U.S.A.) and Cintra 40 (GBC, Australia) spectrophotometers. Operational parameters were: scan speed, 2 nm/s, slit width, 0.3 nm, photometric sensitivity, 0.2 abs. units. Matching pair of 1 and 0.1 cm quartz cuvettes was used for measuring the spectra.

ESI-mass spectrometry was performed on Agilent (Waldbronn, Germany) LC/MSD system operating in FIA mode. ^{1}H- and ^{13}C-NMR spectra of fluoroquinolones and their metal complexes were recorded on a Varian (U.S.A.) Gemini 200 spectrometer. IR spectra were taken on Perkin Elmer model 983G spectrophotometer. The samples were processed as KBr pellets. Scanning electron microscope photographs were taken on JSM 841 A microscope using resolution of 4 nm and magnification of 300k. Thermal analysis was performed on Perkin Elmer DSC 7 analyzer and Perkin Elmer TGS2 thermo-gravimetric balance.

Procedure All titrations were performed in a double mantled, thermostated glass vessel closed with Teflon stopper. A constant temperature, to (298.0±0.1) K, was maintained by circulating thermostated water through the jacket. Purified and oxygen free nitrogen gas was bubbled through the solution for providing an inert atmosphere and stirring. Additional stirring of the solution was achieved with a magnetic stirrer.

To reduce the concentration of the hydrogen ion, the alkali was added stepwise from an autoburette in small aliquots (0.005—0.01 ml). The potential was monitored after each addition of titrant. The titration protocol was chosen in such a way that the hydrolysis and complexation reactions would proceed in the conditions as close to true equilibrium as possible. To achieve this, the potential readings were taken every 2 min until steady values to ±0.1 mV/min were obtained. Hence, the average equilibration time for each point was 5 min at the beginning of titration and 10 min when complexation occurred. If stabilization of potential readings could have not been achieved within this time interval, addition of new aliquot of titrant was initiated and corresponding point was excluded from calculations. No back titrations were performed. Instead, agreement between duplicate titrations (better than 1%) served as a criterion for reversibility of the reaction. The titrations were ter-

minated when drifted potential readings were obtained and turbidity of solutions observed.

Spectral measurements were made on solutions in which the concentration of aluminum and quinolone were constant (C_{Al}=0.025 and 0.05 mmol dm^{-3}, C_{Qln}=0.05 and 0.125 mmol dm^{-3}) while pH was varied between 4.0 and 9.0 (16 solutions). The pH of the test solutions was measured with glass-calomel electrode couple, which was calibrated as a hydrogen concentration probe according to procedure of Irving *et al.*[24] The pH of each test solution was checked daily, during one week. The stable values, within 0.01 pH and 0.004 absorbance units, were attained after 1 h and remained stable during couple of days. Spectra of the test solutions were recorded in 220—400 nm wavelength interval.

For ESI-MS measurements aluminum–quinolone solutions were prepared in the quinolone to aluminum concentration range 1 : 1 and 2 : 1 in the pH range 2.0 to 7.0 and total aluminum concentration 0.50 mmol dm^{-3} and 0.8 mmol/dm^3. The solutions were allowed to stand for 2 d before final adjustment of the pH was made. The spectrometer was operated in FIA mode and the injected volume was 10 μl. Since the nature of the resulting mass spectrum is dependent on various parameters the operational conditions were varied until the best reproducible results were obtained. The capillary voltage was 3.2 or 4.0 kV, sample cone (fragmentor) voltage was either 20, 50, 70 or 100 drying gas flow 10 or 7 l/min, drying gas temperature, 350 °C, solvent (water : methanol, 50/50 v/v) flow 0.3 or 0.05 ml/min, mass range *m*/*z* 0—250 and 50—700. The best conditions were capillary voltage 3.2 kV, cone voltage, 70 V and solvent flow 0.3 ml/min. All ESI mass spectral data in the positive ion mode were acquired and processed using HP ChemStation software.

NMR spectrometer was operating at 200 MHz for proton. In every measurement D_2O was used as a solvent and the pD was regulated by the microliter addition of DCl or NaOD solutions. Thus all solutions had a constant final pD. Each of the metal complex was prepared in an NMR sample tube by the addition of a D_2O solution of $AlCl_3$ into D_2O solution of the ligand. Typical conditions for ^{1}H-NMR measurements were: spectral width 2500 Hz, pulse delay time 1 s, no of scans 128. Chemical shifts (δ, ppm) were obtained for 3-trimethylsilylpropionic acid-d_4 sodium salt as an internal standard (δ=0.000 ppm). ^{13}C-NMR spectra were recorded with proton broad band decoupling and referenced to TMS as an internal standard. For ^{13}C-NMR spectra the spectrometer was operated at 50 MHz. Typical conditions were spectral width 15000 Hz, pulse delay 0, no of scans 50000.

Data Treatment The species formed in the studied systems were characterized by the general equilibrium:

$$p\mathrm{Al}+q\mathrm{H}+r\mathrm{Qln}=\mathrm{Al}_p\mathrm{H}_q\mathrm{Qln}_r$$

and the corresponding constants are given by:

$$\beta_{p,q,r}=\frac{[\mathrm{Al}_p\mathrm{H}_q(\mathrm{Qln})_r]}{[\mathrm{Al}]^p[\mathrm{H}]^q[\mathrm{Qln}]^r}$$

where Qln is the deprotonated molecule of the ligand. Fully protonated quinolone is denoted as H_2Qln^+.

Three kinds of equilibria should be considered in the present study: (a) protonation of quinolone anion; (b) hydrolysis of aluminum(III) ion; and (c) general three component equilibria, which include the case q=0, *i.e.* the formation of pure binary complexes of Al^{3+}. Negative values of q denote hydroxo complexes. The overall protonation constants of quinolone anions and stability constants of hydrolytic complexes of aluminum(III) ion were determined in separate experiments. Thus, in evaluation of three component equilibria (c), the binary models (a) and (b) were considered as known. The concentration stability constants of complexes, $\beta_{p,q,r}$ were calculated with the aid of the suite of computer programs Hyperquad2006.[25] In Hyperquad calculations the identity and stability of complexes which give the best fit to the experimental data, were determined by minimizing the error squares sum of the potentials, U: $U=\sum w_i(E_{obs}-E_{calc})^2$ where w_i represents a statistical weight assigned to each point of titration curve, E_{obs} and E_{calc} refer to the measured potential of the cell and the calculated one assuming the specific model and trial constants, respectively. Quality of fit was judged by usual statistical parameters[25]: Pearsons' test, χ^2, standard deviation in potential residuals, s, and the difference between experimentally determined and calculated standard EMF of the cell. If this difference was higher than 1 mV the titration was discarded. The spectrophotometric data were evaluated with the aid of the program Squad[26] and the program Hyperquad which is capable to treat spectral data. In Squad calculations, the composition, stability and molar absorptivities, $\varepsilon_{p,q,r}$ of complexes, were determined by minimizing the sum, S, defined as: $S=\sum(A_{obs}-A_{calc})^2$ where A_{obs} and A_{calc} refer to measured absorbance and that calculated according to equation: $A_{calc}=\sum\beta_{p,q,r}[\mathrm{Al}]^p[\mathrm{H}]^q[\mathrm{Qln}]^r\varepsilon_{p,q,r}$. Acceptance criteria for each particular model were: S lower than 1.0×10^{-2}, standard deviation of the fit of the spectrum (SD) less than 1.0×10^{-2} and standard deviation in calculated stability constants less than 0.08 log units. For Squad calculations the spectra were digitized at 3 nm intervals. Potentiometric and spectrometric data were made consistent by concomitantly evaluating both kind of data with the aid of Hyperquad 2006[25] suite of programs using the best model obtained in separate treatment. The final model obtained in particular Hyperquad and Squad calculations was optimized. All calculations were performed on an IBM PC compatible computer.

Results and Discussion

In order to study speciation in three-component system Al^{3+}–H^+(or OH^-)–quinolone, it is necessary first to characterize the binary equilibria, *i.e.* hydrolysis of aluminum(III) ion and protonation of quinolone anions, under exactly the same experimental conditions as for complexation study. Hydrolysis of aluminum was studied in our previous work,[23] so the results obtained there along with the literature data[27] were used in this work.

Protonation of Quinolones Anion Protonation constants, β_n, of fleroxacin and moxifloxacin anions, defined according to equilibrium:

$$n\mathrm{H}^+ + \mathrm{Qln}^- \rightarrow \mathrm{H}_n\mathrm{Qln}\,;\quad \beta_n\ (n=1,2)$$

were determined by glass electrode potentiometric titrations in 0.1 mol dm^{-3} LiCl medium at 298 K. Three titrations were carried out with 0.25, 0.50 and 1.10 mmol dm^{-3} total fluoroquinolone concentrations, in the pH range between 3.0 and 10.2. In total 290 points were included in calculations for each quinolone. Spectrophotometric measurements were made on solutions in which the concentration of fluoroquinolone was the same (0.05 mmol dm^{-3}) while the pH values were varied between 4.0 and 9.4 (15 solutions were used). The calculated values of protonation constants are given in Table 1. Agreement between potentiometrically and spectrophotometrically obtained values was better than 1%. The obtained values are in the range with previously reported data.[10,28] Protonation constant K_1 refers to protonation of tertiary nitrogen in piperazinyl substituent at position 4′ in fleroxacin molecule and secondary nitrogen at position 2′ in moxifloxacin (Chart 1) while protonation constant K_2 refers to protonation of carboxylate group. The protonation constants K_2 are considerably higher in the presence of SDS thus leading to conclusion that SDS suppresses the first dissociation of both quinolones. An explanation can be given on the basis of structural characteristics of quinolones. They both contain two polar groups (carbonyl and carboxyl). In addition the positively charged nitrogen (H_3C–N in fleroxacin, HN in moxifloxacin) in the substituent at C-7 constitutes an-

Table 1. Potentiometrically and Spectrophotometrically Determined Protonation Constants of Fleroxacin (Flero) and Moxifloxacin (Moxi) Defined as: K_1=[HQln]/[H^+][Qln^-], K_2=[H_2Qln]/[HQln][H^+]

	Systems			
	Flero	Flero+SDS	Moxi	Moxi+SDS
log K_1	8.06±0.01	8.07±0.05	9.32±0.01	9.35±0.06
log K_2	5.67±0.03	6.60±0.05	6.35±0.02	7.45±0.07

Experimental conditions: 0.1 mol/dm^3 LiCl ionic medium, T=298 K. SDS denotes sodium dodecylsulfate, C_{SDS}=12.0 mmol/dm^3.

Table 2. Experimental Conditions for Potentiometric Titrations in 0.1 mol/dm^3 LiCl Ionic Medium at 298 K

No	C_{Al}	C_L	C_H	C_{SDS}	L/M	pH region	Z_{Al}
Al–fleroxacin							
1	1.40	0.8	4.20	—	0.6	2.364—4.579	0.57
2	0.73	0.8	4.60	—	1.1	2.376—4.679	0.94
3	0.50	0.8	4.60	—	1.6	2.399—6.935	2.49
4	0.70	3.487	2.737	—	5.0	2.998—7.799	2.50
5	1.40	0.8	3.63	12.0	0.6	2.433—4.342	0.65
6	0.73	0.8	3.22	12.0	1.1	2.587—6.851	1.48
7	0.50	0.8	3.00	12.0	1.6	2.589—7.732	2.53
Al–moxifloxacin							
8	1.00	1.28	2.13	—	1.3	2.800—8.558	2.74
9	0.80	1.07	1.74	—	1.4	2.895—8.497	2.88
10	0.52	1.07	1.60	—	2.1	2.978—8.920	4.70
11	0.554	2.503	2.19	—	4.5	3.081—9.000	2.25
12	1.00	5.00	4.381	—	5.0	2.890—9.128	2.20
13	1.00	1.28	1.90	12.0	1.3	2.860—9.303	3.43
14	0.80	1.07	1.39	12.0	1.4	3.058—8.813	2.94
15	0.52	1.07	1.39	12.0	2.1	3.168—9.043	2.55

C_X is the analytical concentration of the corresponding species in mmol/dm^3, L denotes ligand and M metal. Maximal average ligand number is denoted as Z_{Al}.

other polar site in the molecule. These groups can cause an electrostatic attraction with the polar head of SDS molecule thus expelling water molecules around the cation and hence impeding the proton transfer from cation to bulk water. Splitting off the first proton (from carboxyl) group is hindered because the hydrogen bond between the carboxyl proton and carbonyl oxygen is stabilized in the presence of SDS. The interaction between N^+ and dodecylsulfate anion is less pronounced than that with carboxyl group and hydrophobic parts of the molecule.

Potentiometric Measurements The experimental data obtained by emf measurements in 0.1 mol dm^{-3} LiCl medium at 298 K are summarized in Table 2.

Both, moxifloxacin and fleroxacin are only sparingly soluble in water, especially at pH values around their isoelectric point. Thus to obtain acceptable parameters of potentiometric titration curves[29] two sets of titrations were performed. First set involved only solutions with lower concentration ratios of ligand to metal (L/M$\leq$2) and the second set involved higher concentration ratios of L/M up to 5.0. Solutions for the first set of titrations were obtained by mixing appropriate volumes of standard solutions of ligand and metal ion. For the second set of titrations calculated amount of solid re-crystallized $AlCl_3$ and solid pure ligand were mixed and appropriate volume of water was added. Moxifloxacin+$AlCl_3$ mixture immediately produced clear solution while with fleroxacin a slurry with insoluble material was obtained. However, upon addition of standard HCl into the slurry (pH *ca.* 3) a clear solution was obtained. Al–moxifloxacin solutions could be titrated up to pH *ca.* 8.5 (L/M$>$2) without appearance of any significant turbidity while Al–fleroxacin solutions remained clear up to pH *ca.* 6.5 when slight opacity was observed but at pH values around 7.5 the solutions again became clear and remained clear up to pH *ca.* 8. Titration points obtained in opaque solutions were discarded in calculations. The establishing of equilibrium in solutions was moderately slow especially at the pH values higher than 6.0. The equilibrium potential was established faster in solutions with higher ligand to metal concentration ratios.

In order to derive the speciation model for each studied system the experimental data were plotted as the dependence of pH on titration parameter. The titration parameter, a, was calculated through the formula

$$a = \frac{BV_B - V_0[\mathrm{HCl}]}{V_0 L}$$

where B and V_B denote concentration and volume of added strong base (NaOH), respectively, while V_0 and L are the initial volume and concentration (quinolone) of the titrated solution. Negative values of a represent the titration of excess of strong acid (HCl). Titration curves of quinolones in the presence of aluminum ion (Figs. 1, 2) are shifted to the right comparing to the titration curve of quinolones alone thus indicating the complex formation in the system. Since the titration curves of quinolone alone and Al^{3+}+quinolone do not coincide at low pH values it may be inferred that complexation reaction proceeds even at pH values lower than *ca.* 3. Coincidence of the titration curves of Al^{3+}+quinolone with different ligand to metal concentration ratios in the pH region around 3 indicates the formation of the 1 : 1 complexes. The titration curve of quinolone alone shows two well separated jumps indicating the titration of two protons from the ligand. In the presence of aluminum ion these protons are titrated at lower pH values and appearance of two buffer regions on the titration curves points to formation of the complexes with ligand to metal ratio higher than 1 : 1. The titrated protons may arise either form coordinated water molecules of from second ligand molecule or both. Thus, it may be expected the formation of complexes with the stoichiometry L/M=1 : 1 and 2 : 1 as well as mixed complexes.

In the presence of SDS titration curves (not shown) are similar but are shifted toward higher pH values for corresponding values of the titration parameter. Titrations with higher ligand to metal concentration ratios were not performed in the presence of SDS due to intensive flocculation in the systems.

Initially the following (p, q, r) complexes were selected to find the model which best fit the experimental data: $(1, 0, 1)$; $(1, 0, 2)$; $(1, 1, 1)$; $(1, 2, 1)$; $(1, 1, 2)$; $(1, -1, 1)$; $(1, -2, 1)$; $(1, -3, 1)$; $(1, -1, 2)$; $(1, -2, 2)$; $(1, -2, 3)$ and polymers $(2, 1, 1)$; $(2, 2, 1)$; $(2, 1, 2)$; $(2, -1, 1)$; $(2, -2, 1)$; $(2, -2, 2)$; $(2, -3, 1)$; $(2, -3, 2)$; $(3, -1, 1)$; $(3, -2, 1)$; $(3, -1, 2)$;

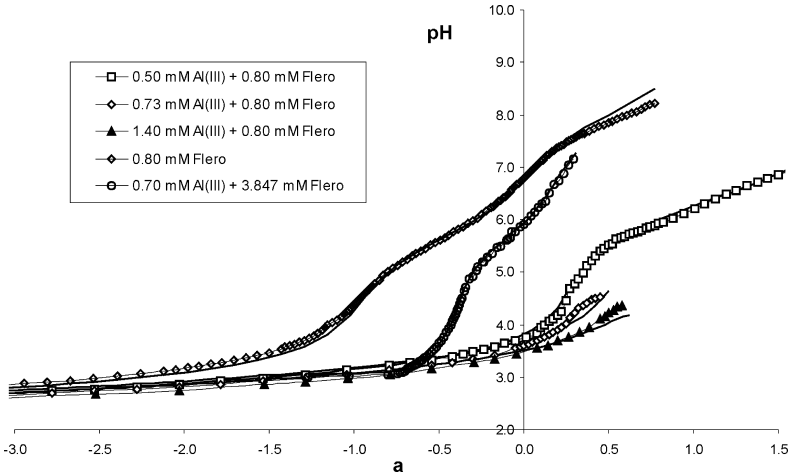

Fig. 1. Potentiometric Titration of Al–Fleroxacin Solutions with NaOH in 0.1 mol/dm^3 LiCl Ionic Medium at 298 K
Full lines denote calculated curves using the data from Table 3. The concentration in mmol/dm^3 is denoted as mM. Flero denotes fleroxacin.

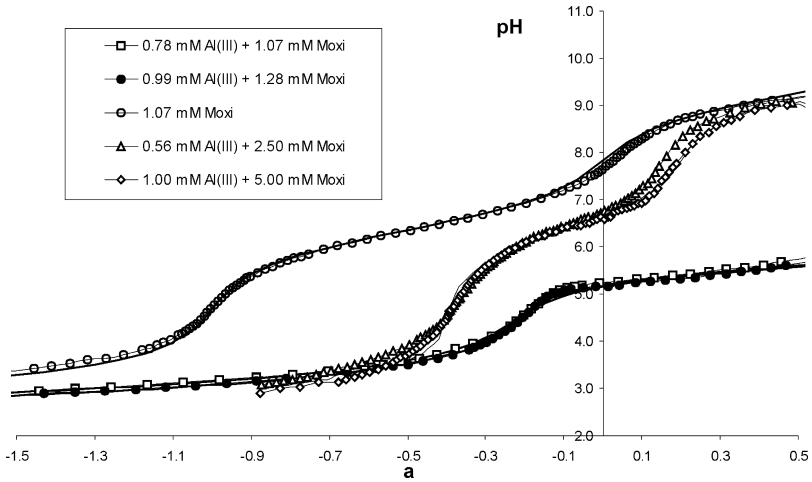

Fig. 2. Potentiometric Titration of Al–Moxifloxacin Solutions with NaOH in 0.1 mol/dm^3 LiCl Ionic Medium at 298 K
Full lines denote calculated curves using the data from Table 3. The concentration in mmol/dm^3 is denoted as mM. Moxi denotes moxifloxacin.

$(3,-2,2)$. More than 20 various models were tested. During the calculations analytical parameters (M_0, H_0 and L_0) were held constant while E_0 values were allowed to float. The pure hydrolytic complexes and protonated quinolone species were not refined during the calculations. The refinement operations for each total aluminum concentration (M) resulted in different and often acceptable models. Different strategies were employed in the refinement operations: (i) fixing selected constants to simplify optimization procedure, (ii) reducing the number of experimental points included in calculations, (iii) "piecewise" fitting of the experimental data. At total ligand to aluminum concentration ratios lower than 2 finally derived model contained the $(1,1,2)$, $(1,1,1)$, $(1,0,1)$, $(1,-1,1)$ and $(1,-2,1)$ complexes. No polynuclear mixed complexes were found. The finally derived model was used to simulate titration curves with L/M$\leq$2. Overall relative standard deviation of the fit was about 2% so that fit can be regarded as good. At higher ligand to aluminum concentration ratios we started optimization by fixing the values of the constants obtained in the previous cycle and searched for the complexes with ligand to metal ratio higher than 2. The following complexes were introduced into the model: $(1,0,2)$; $(1,1,2)$, $(1,2,2)$, $(1,0,3)$ and $(1,1,3)$. The results of optimization showed that the complexes with ligand to aluminum ratio three are always excluded from the model. Thus we narrowed the search only to L/M=2. The complexes $(1,0,2)$ and $(1,1,2)$ could be accepted in the model but with rather poor statistical parameters of the fit. Excluding the complex $(1,1,2)$ from the model did not improve the fit significantly. However, excluding the complex $(1,0,2)$ and keeping $(1,1,2)$ dramatically improved the fit. Therefore, we included the complex $(1,1,2)$ in the accepted model. In the final calculation cycle we used all the data points in the pH range 3 to 7 for fleroxacin and 3 to 8.5 for moxifloxacin with the model $(1,1,2)$, $(1,1,1)$, $(1,0,1)$, $(1,-1,1)$ and $(1,-2,1)$ and fixing the value of the stability constant of the $(1,1,1)$ complex. This species was firmly confirmed in a pre-optimization procedure but strongly competes with $(1,0,1)$ complex at lower pH values. Upon increasing the pH the complex AlL undergoes successive deprotonation and thus its concentration lowers. So in competition with AlHL it may readily go undetected and this was the reason why we fixed the constant of AlHL. When we established the constant of AlL we allowed the constant of AlHL to float and it remained practically un-

changed. The calculation produced good fit with acceptable set of statistics. To examine the sensitivity of this model we co-varied the stability constant of pure hydrolytic Al_{13}-mer with the constants of the complexes from accepted set introducing these species into the optimization procedure one by one. The calculated values of the stability constants remained practically the same. It means that the derived model is robust and involves all main species formed in solution.

The results of calculations indicated that the main species in aluminum–quinolone solutions at lower ligand to aluminum concentration ratios and lower pH values (pH <3) is the complex Al(HQln) while at higher aluminum concentrations and intermediate and higher pH values the main complex is Al(HQln)Qln complex. Mixed hydrolytic complexes are not important even at higher pH values.

Spectrophotometric Measurements Spectral measurements were performed on Al^{3+}–quinolone solutions in which the concentration of both, aluminum ion and quinolone was kept constant while pH was varied by the addition of the standard HCl or NaOH, as appropriate. The spectra show evidence of an intensive band centered at 280 and 290 nm in Al–fleroxacin and Al–moxifloxacin systems respectively. Another lower energy broad band appears between 300 and 360 nm for Al–fleroxacin solutions and between 320 and 380 nm for Al–moxifloxacin solutions. This band shows ill defined maximum at 330 nm for Al–fleroxacin and two well resolved maxima at 340 and 380 nm for Al–moxifloxacin solutions (Fig. 3). The high energy band is mainly due to the $\pi\rightarrow\pi^*$ transition in the aromatic ring. The low energy band is due to $n\rightarrow\pi^*$ transition in diazabicyclo substituent at position C-7 for moxifloxacin and in piperazyne substituent at C-7 (Chart 1) for fleroxacin, and consists of two subpeaks. These subpeaks also reflect the participation of non-bonding electron pair on nitrogen at position 1 and are caused by an intermolecular hydrogen bond equilibrium between moxifloxacin and water as well as intramolecular hydrogen bond between 4-keto and 3-carboxylic groups.[30—32] Upon increasing the pH from *ca.* 4 to 9 higher energy band shows only small changes in position and maximum intensity (hypsochromic shift). The lower energy band exhibits however, significant changes in a shape, position and intensity (bathochromic shift). At pH values lower than 7, this band is fairly symmetrical with a shoulder at 350 nm, but at pH values higher than 7 two separated absorption maxima at 335 and 355 nm are obtained for moxifloxacin solutions. Fleroxacin solutions produce less separation of the sub-peaks. Intensity of the low energy band increases upon increasing the pH up to *ca.* 8 and then decreases. In the presence of aluminum ion, in comparison with the spectrum of quinolones alone, all bands are shifted toward higher wavelengths (batochromic shift) for *ca.* 10 nm for Al–fleroxacin and *ca.* 20 nm for Al–moxifloxacin systems. The spectra of aluminum–quinolone solutions were not taken in the presence of SDS due to turbidity of the systems unsuitable for spectral measurements.

The spectral data were first evaluated with the aid of the Squad program. In calculations, the molar absorptivities of quinolne anion, H(Qln) and H_2(Qln) were known from spectral measurements of quinolone anion protonation and were fixed, while these of aluminum(III)-aqua ion and pure hydrolytic complexes were set to zero. The calculations were

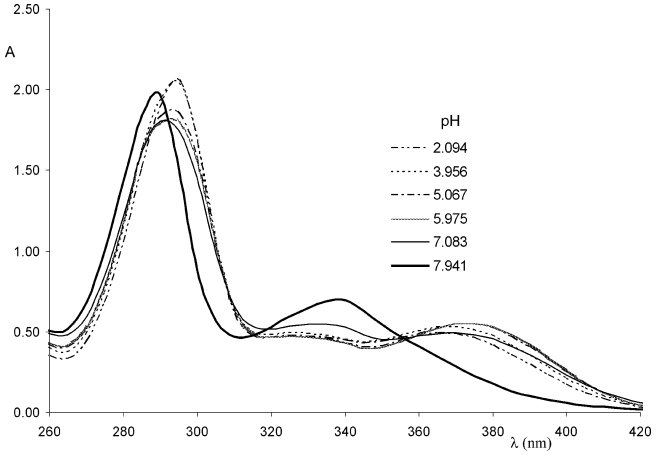

Fig. 3. The UV–Vis Spectra of Al^{3+}–Moxifloxacin Solutions at Different pH Values

L/M=2.0.

carried out in the following way: the complexes found by potentiometry were included in Squad calculations and their stability constants were allowed to float. When the best fit of the spectra was achieved the stability constants were varied one at a time simultaneously with variation of molar absorptivities. The final calculation cycle was performed with Hyperquad 2006 suite of programs where selected potentiometric and spectrophotometric data were used altogether. The best model, from both particular calculations, was included in Hyperquad calculations with the trial set of stability constants. First, manual option of Hyperquad was used which produced agreement of about 5% between experimental and calculated curves. The trial set of stability constants was finally optimized until calculated statistical parameters achieved acceptable values (standard deviation of the fit $<10^{-2}$, standard deviation in residuals $<10^{-3}$). The finally accepted results of calculation are given in Table 3.

Along with the stability constants, in spectral calculations, the molar absorptivities of the complexes were calculated. The calculated spectra for Al–moxifloxacin system are presented in Fig. 4. As seen from Fig. 4 the calculated spectra of Al(HMoxi) and Al(HMoxi)Moxi complexes resemble these of HMoxi and Moxi while the spectrum of AlMoxi complex differs from that of pure HMoxi most significantly in the region of $n\rightarrow\pi^*$ transition in the 350—430 nm wavelength interval. It is probably caused by breaking the intra- and intermolecular hydrogen bonds due to coordination of both 4-keto and 3-carboxyl oxygens to aluminum.

The distribution of various complexes in Al–moxifloxacin solution is shown in Fig. 5. At pH values lower than 4 the dominating complex is $Al(HMoxi)^{2+}$ which upon increasing the pH releases proton and gives the binary complexes $Al(Moxi)^{2+}$. The formation of the binary complex is a great deal suppressed by parallel formation of mixed complex $Al(HMoxi)Moxi^{2+}$ which proceeds in a wide pH range from 3 to 8. Depending on concentration ratio of Moxi to Al and total Al concentration at pH values higher than 6 the formation of soluble $Al(OH)_{3(aq)}$ and aluminate, $Al(OH)_4^-$ begins. The formation of insoluble $Al(OH)_{3(s)}$ starts at pH *ca.* 7. The concentration of this complex and aluminate steeply increases with increasing the pH. Mixed hydrolytic complexes are only minor species and become significant at L/M<1 and

Table 3. Stability Constants of Aluminum–Fluoroquinolone Complexes Formed in a 0.1 mol/dm^3 LiCl Ionic Medium, at 298 K in the Absence and in the Presence of 12.0 mmol/dm^3 Sodium Dodecylsulfate (SDS)

Species (p, q, r)	$\log \beta_{p,q,r}$			
	Al(III)–Flero	Al(III)+SDS+Flero	Al(III)–Moxi	Al(III)+SDS+Moxi
$Al(HL)L^{2+}$	24.87 (6)	—	28.47 (5)	—
$Al(HL)^{3+}$	14.02 (3)	16.20 (6)	16.59 (4)	17.45 (5)
AlL^{2+}	11.41 (1)	11.62 (2)	11.66 (1)	12.49 (5)
$Al(OH)L^{+}$	5.20 (2)	5.25 (3)	5.28 (2)	6.05 (3)
$Al(OH)_2L$	−1.61 (2)	—	−2.92 (2)	—
χ^2	11.40	11.20	12.40	12.85
S	1.6	1.27	1.5	1.2

Titrations with L/M>2 were not performed in the presence of SDS. Standard deviation in calculated constants are given in parenthesis. Flero=fleroxacin, Moxi=moxifloxacin.

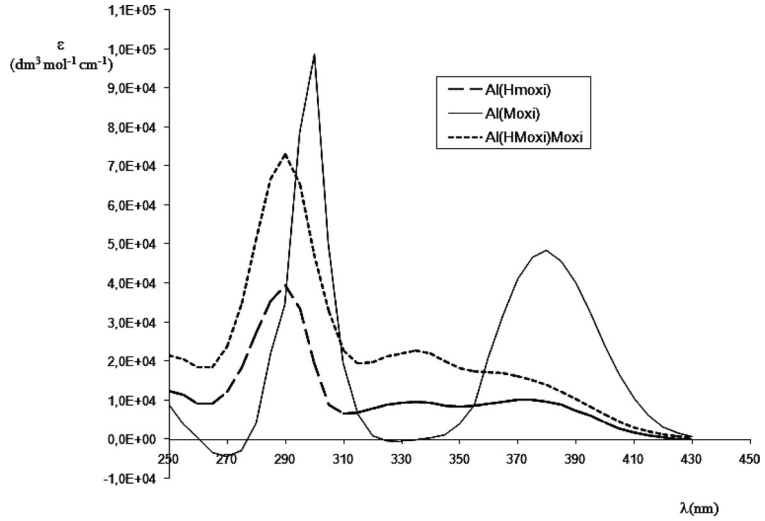

Fig. 4. The Calculated Spectra of Al–Moxifloxacin Species

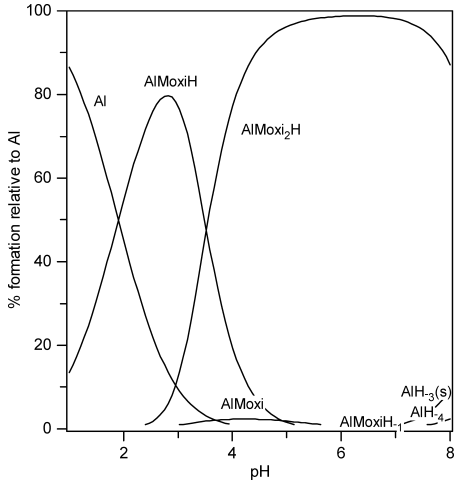

Fig. 5. The Overall Distribution Diagram of Al–Moxifloxacin Species at Ligand-to-Metal Concentration Ratio=2 and Total Aluminum Concentration 1.0 mmol/dm^3

total Al concentration less than 1.0 mmol/dm^3.

The distribution of the complexes in Al–moxifloxacin system at low pH values from pH *ca.* 1 to 3 shows that the dominating complex is $Al(HMoxi)^{3+}$ which bears the charge +3 and thus can not be transported by *trans*-cellular route owing to hindrance to cross the lipid barrier. These conditions correspond to those in gastric juice.

The distribution of complexes at higher pH values mimicking those in duodenum reveals that the complexes $Al(OH)Moxi^{+}$ and $Al(OH)_2Moxi^{0}$ may reach the appreciable concentration and since the complex $Al(OH)_2Moxi$ is not charged it may cross the cell membrane lipid barrier and thus increase Al toxicity. Though the concentration of $Al(OH)_2Moxi$ complex is low in comparison with insoluble hydroxide and aluminate one may bear in mind that insoluble aluminum species are adsorbed on intestinal mucus and thus excreted by intestinal motility. This may shift the equilibrium conditions toward increasing the concentration of the neutral soluble complex and thus lead to transportation of soluble aluminum into intestinal capillaries. Under high long-term therapeutic doses of antacids it may lead to expression of aluminum toxicity.

In Al–fleroxacin system the relative concentration of the species are similar to that in Al–moxifloxacin system. In the presence of SDS at L/M<2 the distribution changes and the dominating complex is Al(HQln) in the pH range 3—5. The distribution of mixed hydrolytic complexes are also changed *i.e.* there was no evidence of $Al(Qln)H_{-2}$ complex formation in the presence of SDS. In the Al–moxifloxacin+SDS system the distribution is similar to that in Al–fleroxacin+SDS system though SDS exerts less influence on Al(HMoxi) formation than on the Al(HFlero). Though the complex

Table 4. Chemical Shifts (ppm) for Fleroxacin (Flero) and Fleroxacin/Al(III)=1 : 1.2 at 25 °C and pH=2.5 in D_2O

Carbon atom	^{13}C		^{1}H	
	[Flero]	[Flero]/[Al] (1 : 1)	[Flero]	[Flero]/[Al] (1 : 1)
C-4	177.6	177.4		
C-11	171.2	172.2		
C-2	155.2	158.5	8.77 (s)	9.03 (s)
C-5	110.2	112.4	7.87; 7.92 (dd)	8.12; 8.19 (dd)
C-12	63.4	64.0	5.0—5.2 (m)	
C-13	84.1	84.6		
C-1′	56.4	58.2	3.5—3.9 (m)	
C-4′	56.4	58.2		
C-2′	50.1	51.9	3.2—3.4 (m)	
C-3′	50.1	51.9		
C-14	46.1	47.6	2.95 (s)	
C-3	119.1	126.1		
C-6	158.5	162.5		
C-7	138.1	137.8		
C-8	148.1	148.5		
C-9	131.0	132.2		
C-10	119.0	125.9		

Table 5. Chemical Shifts (ppm) for Moxifloxacin and Moxifloxacin+Al(III) at 25 °C and pH 4.5 in D_2O

Carbon atom	^{13}C			^{1}H		
	[Moxi]	[Moxi]/[Al] (1 : 1)	[Moxi]/[Al] (1 : 2)	[Moxi]	[Moxi]/[Al] (1 : 1)	[Moxi]/[Al] (1 : 2)
C-4	178.5	176.9	177.6			
C-11	172.3	173.5	173.1			
C-2	153.4	156.9	156.9	8.8 (s)	9.1 (s)	9.0 (s)
C-5	103.4	111.8	111.7	7.2 (d)	8.0 (d)	7.9 (d)
C-12	64.7	65.2	66.2	3.6 (s)	3.6 (s)	3.6 (s)
C-13	58.5	60.1	60.1	2.8—4.4 (m)		
C-9′	57.0	59.0	58.9			
C-7′	54.6	55.9	55.6			
C-3′	45.6	47.3	47.2			
C-1′	44.3	46.1	46.1			
C-6′	37.4	38.8	38.8	1.8—2.0 (m)		
C-4′	23.5	24.7	24.7			
C-5′	20.4	21.6	21.6			
C-15	12.4	13.8	13.7	0.9—1.4 (m)		
C-14	11.2	12.5	12.4			

$Al(Moxi)H_{-2}$ does not form in the presence of SDS, the formation of pure hydrolytic complexes of aluminum becomes significant at pH values higher than 5 and increases upon increasing the pH. In base medium the formation of soluble or micro-colloidal $Al(OH)_3$ is pronounced and this complex is probably the pre-cursor of the precipitate which finally forms. To examine the chemical nature of the precipitate formed, at the end of the titrations the precipitate was collected by centrifugation. The precipitate was thoroughly washed with water and methanol and subsequently air dried. TG analyses indicate presence of weakly (*ca.* 80—110 °C) and strongly (*ca.* 250 °C) bound water. The infrared spectrum (3430 cm^{-1} broad-strong, 1700 cm^{-1} medium sharp, 1450 cm^{-1} weak, 1000 cm^{-1} broad weak, 625 cm^{-1} broad medium bands) corresponds to hydrated aluminum oxide. The SEM photographs of the precipitate indicate its amorphous character. Thus, it may be concluded that the precipitate is micro-colloidal aluminum hydroxide. Similar result was found by Drevenšek *et al.*[33] in magnesium–ciprofloxacin system where the formation of micro-colloidal magnesium hydroxide was found.

NMR Measurements The ^{1}H- and ^{13}C-NMR spectral assignments for fleroxacin and moxifloxacin are given in Tables 4 and 5 respectively. The assignments were made according to literature data.[18,34—36] The binding of fluoroquinolones to Al^{3+} was studied by changing the pD of solutions with constant ligand-to-metal conc. ratio and constant concentration of ligand and by changing of ligand to metal molar ratio while holding the concentration of the ligand and pD of solution constant.

Studies using Al^{3+} resulted in the appearance of additional peaks with the addition of metal ion to the ligand solution due to slow exchange between complexed and free ligand. When Al^{3+} was added the proton spectra of fleroxacin (Fig. 6) showed additional signals for each signal in the free ligand spectrum. The C-2 and C-5 signals show most pronounced changes. In addition to C-2 signal at 8.77 (s) ppm, new signal appears at 9.03 (s) ppm while C-5 doublet–doublet signals (due to coupling with fluorine) at 7.87 and 7.92 (dd) produce new signals at 8.12 and 8.19 (dd) ppm. Upon raising the

pH from 2.5 to 4.5 appearance of new signals at 8.32 and 8.38 ppm indicate formation of a new complex. Increasing the concentration ratio of fleroxacin to Al does not change shifts at C-2 and C-5 appreciably. Addition of Al^{3+} into the drug solution induced a great change in the ^{13}C spectrum. The signals due to the free ligand decrease in intesites and some new signals appeared as the amount of Al^{3+} or pD increased. The changes are seen on C-4 and C-2 atoms where the signals shift from 177.65 to 177.4 ppm and 155.2 to 158.5 ppm respectively.

The proton spectra of moxifloxacin in D_2O show the C-2 and C-5 protons from quinolone nucleus at 8.8 (s) and 7.2 (d) ppm, respectively. Upon addition of Al^{3+} these protons undergo the most appreciable changes. The C-2 gives new signal at 9.1 ppm while C-5 appears at 8.0 ppm.

In ^{13}C spectrum of Al^{3+}+moxifloxacin in comparison with pure moxifloxacin spectrum the distance between carbonyl C-4 and carboxyl C-11 signals decreases which indicates the deprotonation of carboxyl group and clearly confirms binding of Al to carboxyl and carbonyl oxygens. Thus, NMR data confirm bidentate binding of both quinolones to aluminum.

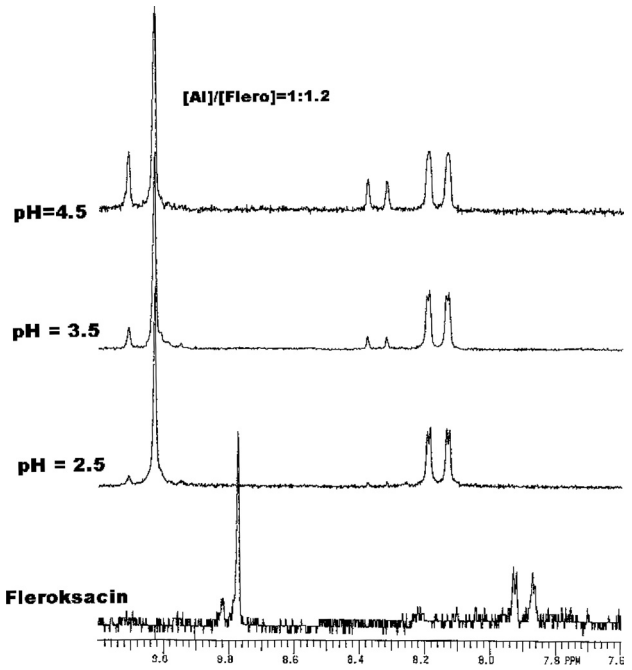

Fig. 6. ^1H-NMR Spectra of Al–Fleroxacin Solutions with Varying pH Values

ESI-MS Measurements The ESI-MS data on pure 10 μmol/dm^3 solution of $AlCl_3$ (pH=4) gives multitude of lines of low intensity which indicate the formation of large number of polynuclear species.[37] The spectrum of moxifloxacin hydrochloride at pH 2.0, 4.0, 7.0 and 9.0 consists only of two lines with m/z=402.2 and 403.2 which corresponds to base ion and indicates absence of fragmentation under the experimental conditions chosen.[38] The spectra of Al^{3+}+moxifloxacin solutions with moxifloxacin to Al concentration ratio 1.2 : 1 and pH values 3.0, 4.0 and 5.0 show very little fragmentation with the signals (in addition to moxifloxacin base peak) at m/z=251.7, 276.6 and 414.2 (Fig. 7). These lines are seen at retention times from 0.3 to 2 min. They may be attributed to the complexes: $[AlCl_2(Moxi)H_2]^{2+}$, $[AlCl_2(Moxi)H_2+MeOH]^{2+}$ and $[Al(Moxi)_2]^+$, respectively. Lines at higher m/z values are of low intensity and are probably artifacts. Analysis of ESI-MS data indicates the formation of bis-complex, $Al(Moxi)_2$ in a wide range of Moxi to Al concentration ratios and pH values. The formation of this complex is consistent with potentiometric data which gave evidence for the formation of the complex $AlH(Moxi)_2$.

Consideration of the experimental data shows that at lower pH values the main complex in Al(III)–quinolone solutions is protonated species $Al(HQln)^{3+}$. Bearing in mind pH range in which it forms, one may suppose that its formation proceeds according to reaction:

$$Al(OH)^{2+} + H_2Qln^+ \rightleftarrows Al(HQln)^{3+} + H_2O$$

Isoelectric point of fluoroquinolones is at pH *ca.* 7.5 so that at pH values lower than 5.0 most fluoroquinolone exists in the cationic form. Reactive species of aluminum at pH between 3.0 and 3.6 is monohydroxo complex $Al(OH)^{2+}$ so that above reaction should be more probable than the one in which aqua-aluminum reacts with neutral fluoroquinolone:

$$Al^{3+} + HQln \rightleftarrows Al(HQln)^{3+}$$

Upon increasing the pH complex Al(HQln) begins to hydrolyze to Al(Qln) complex which upon increasing the pH undergoes further successive deprotonation.

In Al(Qln) complex 3-carboxyl and 4-carbonyl groups are involved in coordination owing to high affinity of aluminum towards oxygen. NMR data are consistent with such structure since the largest chemical shifts are observed for the C atoms in the vicinity of the carbonyl C-atom.

The formation of $AlHL_2$ complex proceeds in a wide range of pH values and bearing in mind that its formation

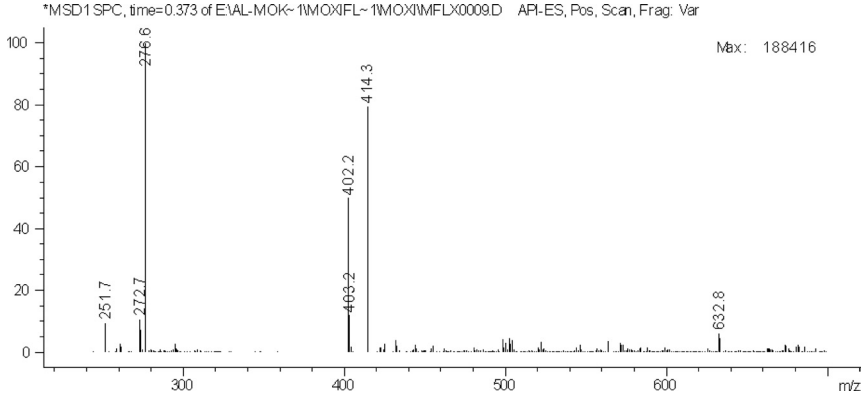

Fig. 7. ESI-MS Spectrum of Al–Moxifloxacin Solution at pH=4.5

starts in acidic medium it most probably forms by reaction:

$$Al(HL)+L \rightleftarrows Al(HL)L$$

The equilibrium constant, K_{eq}, for the above reaction may be calculated from overall stability constants of Al(HL)L and Al(HL) complexes: $\log K_{eq}=\log \beta_{1,1,2}-\log \beta_{1,1,1}$. For Al–moxifloxacin system $\log K_{eq}=28.47-16.59=11.88$ and for Al–fleroxacin, $\log K_{eq}=24.87-14.02=10.75$. These values are comparable with the overall formation constants for the binary AlL complex. This is the reason why the formation of the binary complex is largely suppressed at higher concentration ratios of ligands to aluminum.

Alternative reaction: $Al(L)+HL \rightleftarrows Al(HL)L$ has $\log K_{eq}=\log \beta_{1,1,2}-\log \beta_{1,1,1}-\log \beta_{0,1,1}$ which for moxifloxacin gives: $\log K_{eq}=28.47-16.59-9.32=2.56$ and thus is far less probable than the former one.

Interactions between aluminum ion and fluroquinolones may occur in systemic circulation. Under physiological conditions the concentration of free aluminum ion in plasma is very low because of the availability of numerous biological ligands (transferrin, albumin, citrate, phosphate and hydroxide) forming very stable complexes with aluminum.[39] However, high serum levels of aluminum may be found in patients with chronic renal impairment, who are treated with dialysis fluids that contain Al or are given Al–hydroxide gels to control high plasma level of phosphate. Thus, complexation reactions *in vivo* between aluminum ion and fluoroquinolones should occur as a series of ligand exchange reactions. The complex formation between aluminum ion and fluoroquinolones in plasma is influenced by kinetic effects, competition by other ligands and metal ions and systemic transport kinetics of fluoroquinolones. Chelating effect of fluoroquinolones may affect the aluminum toxicity by mobilizing free Al ion into (mainly) urine. Ca^{2+} and Mg^{2+} are the most important ions competing for fluoroquinolones. Anticipating that AlL complex is excreted in urine the mobilizing power of fluoroquinolones to excrete aluminum may be estimated through equation:

$$E=\frac{\beta_{ML}}{\beta_{CaL}}\times\frac{[L_t]}{[Ca^{2+}]}$$

where β_{ML} and β_{CaL} denote stability constants of 1 : 1 chelates of aluminum and calcium with fluoroquinolones, respectively, $[L_t]$ is total plasma concentration of quinolones and $[Ca^{2+}]$ is concentration of free calcium in plasma.[40] With β_{AlL} *ca.* 10^{11}, β_{CaL} *ca.* 10^2, $[L_t]$ *ca.* 0.01 mmol/l, $[Ca^{2+}]$ *ca.* 2.5 mmol/l the mobilizing power, E is 4×10^6. Important essential ion is magnesium and it is implicated in quinolone binding to DNA.[41] The stability constant of Mg–quinolone complex is about $\log\beta$ *ca.* 3[41] so as the mobilizing power of fluoroquinolones in relation to magnesium ion whose concentration in plasma is *ca.* 1 mmol/l[42] is similar to that calculated in relation to calcium. It is known that fraction of aluminum not bound to transferrin is excreted in kidneys or it may form deposits in kidney tissue. Fluoroquinolones are also excreted in kidneys and thus they may mobilize aluminum excretion into urine[43] by forming positively charged soluble complexes. It seems that fluoroquinolones fleroxacin and moxifloxacin may reduce aluminum toxicity in blood *i.e.* they may exhibit antidotal effect on aluminum in case of increased plasma levels of aluminum ion.

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